Methyl Group Migration During the Fragmentation of Singly Charged Ions of Trimethyllysine-Containing Peptides: Precaution of Using MS/MS of Singly Charged Ions for Interrogating Peptide Methylation

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Core histones are susceptible to a range of post-translational modifications (PTMs), including acetylation, phosphorylation, methylation, and ubiquitination, which play important roles in the epigenetic control of gene expression. Here, we observed an unusual discrepancy between MALDI-MS/MS and ESI-MS/MS on the methylation of trimethyllysine-containing peptides with residues 9–17 from human histone H3 and residues 73–83 from yeast histone H3. It turned out that the discrepancy could be attributed to an unusual methyl group migration from the side chain of trimethyllysine to the C-terminal arginine residue during peptide fragmentation, and this methyl group transfer only occurred for singly charged ions, but not for doubly charged ions. The methyl group transfer argument received its support from the results on the studies of the fragmentation of the ESI- or MALDI-produced singly charged ions of several synthetic trimethyllysine-bearing peptides. The results presented in this study highlighted that caution should be exerted while MS/MS of singly charged ions is employed to interrogate the PTMs of trimethyllysine-containing peptides. (J Am Soc Mass Spectrom 2009, 20, 1172–1181) © 2009 American Society for Mass Spectrometry

The nucleosome core particle consists of a histone octamer around which DNA is wrapped [1]. Two H2A-H2B dimers flank a centrally located $(H3\text{-}H4)_2$ tetramer to give the histone octamer [1]. The core histones have a similar structure with a basic N-terminal domain, a globular domain organized by the histone fold, and a C-terminal tail. Core histone N-terminal tails, which emerge from the core particle in all directions, are involved in the establishment of a spectrum of chromatin structural states, while their histone fold domains mediate histone-histone and histone-DNA interactions [1].

The core histones are susceptible to an array of post-translational modifications (PTMs), including acetylation, phosphorylation, methylation, and ubiquitination [2, 3]. Histone methylation, which occurs on the side chains of lysine and arginine, is the most prominent in histones H3 and H4, and it is associated with transcriptional activation, differentiation, imprinting, and X-inactivation [3, 4]. In general, methylation at H3-K4, H3-K36, and H3-K79 is associated with euchromatin and gene activation, whereas methylation at H3-K9, H3-K27, and H4-K20 is involved with heterochromatin and repressed genes. Moreover, histone methylation, together with acetylation and phosphorylation, can form a histone code to provide a "mark" to recruit downstream chromatin assembly or modification proteins for chromatin remodeling and transcription activation [3, 4].

MALDI-MS/MS and LC-ESI-MS/MS have been widely used for assessing the PTMs of histones [5, 6]. Together with HPLC separation and enzymatic digestion, they can provide detailed information about the modification sites and levels. Understanding peptide fragmentation is important for investigating the PTMs of proteins by MS/MS with the "bottom-up" strategy. In this context, a protein can be digested to peptides, which can be cleaved by surface-induced dissociation (SID) or collision-induced dissociation (CID) at the amide linkages, to afford a series of b and y ions. The b and y ions are thought to arise from a "charge-directed" pathway where cleavage occurs in the vicinity of a charge site [14]. A "mobile proton transfer" model proposed by Wysocki et al. [7–13] is widely accepted for rationalizing the "charge-directed" fragmentation of peptides, where a proton is transferred from the peptide N terminus or side chains to the cleavage site [14].

Here, we observed an unusual difference between MALDI- and ESI-MS/MS when analyzing trimethyllysine-

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containing peptides from the Arg-C digestion of histone H3 isolated from cultured human and yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) cells. It turned out that a methyl group in trimethyllysine could migrate during the fragmentation of singly charged ions of these peptides, but this migration was not observed for the corresponding doubly charged ions. Based on these findings, we advocate that caution should be exerted while MS/MS of singly charged ions is employed for assessing peptide methylation.

Experimental

Trimethyllysine-containing synthetic peptides were obtained from Biomatik (Ontario, Canada), and N^G-monomethyl-L-arginine was obtained from Sigma-Aldrich (St. Louis, MO).

Cell Culture and Protein Extraction

Human HL-60 cells [American Type Culture Collection (ATCC), Manassas, VA] were cultured in RPMI-1640 medium (ATCC) with 10% FBS at 37 °C until the cell density reached between 10^5 and 10^6 cells/mL. Human MCF-7 cells (ATCC) were cultured in DMEM medium (ATCC) with 10% FBS at 37 °C until the cells were at 80% confluence level.

The cells were harvested by centrifugation at 5000 rpm. The cell pellets were subsequently washed with a 5-mL buffer containing 0.25 M sucrose, 0.01 M $MgCl_2$, 0.5 mM PMSF, 50 mM Tris (pH 7.4), and 0.5% Triton X-100. The cell pellets were then resuspended in 5 mL of the same buffer and kept at 4 °C overnight [15]. The histones were extracted from the cell lysis mixture with 0.4 N sulfuric acid by incubating at 4 °C for 4 h with continuous vortexing. The histones in the supernatant were precipitated with cold acetone, centrifuged, dried, and redissolved in water.

Wild-type BY4742 *Saccharomyces cerevisiae* cells (Open Biosystems, Huntsville, AL) were cultured in a medium containing 1% yeast extract, 2% peptone, and 2% glucose. The cells were harvested when the OD_{600} reached between 1 and 2. In this regard, the cells were centrifuged at 5000 rpm at 4 °C for 10 min, and the resulting cell pellets were washed with sterile water and resuspended in a solution bearing 0.1 mM Tris (pH 9.4) and 10 mM DTT. The mixture was incubated at 30 °C for 15 min with gentle shaking. The cells were recovered by centrifugation and the cell pellets were washed with a solution containing 1.2 M sorbitol and 20 mM HEPES (pH 7.4), and centrifuged again. The resulting cell pellets were resuspended in the same buffer (50 mL) containing 20–30 mg zymolyase and incubated at 30 °C for 30 min with gentle shaking to digest the cell wall. Cells were subsequently washed twice with ice-cold nuclei isolation buffer (0.25 M sucrose, 60 mM KCl, 14 mM NaCl, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 15 mM MES, 1 mM PMSF, 0.8% Triton X-100), followed by washing three times with buffer A (10 mM Tris, pH 8.0, 0.5% NP-40, 75 mM NaCl, 30 mM sodium butyrate, 1 mM PMSF) and twice with buffer B (10 mM Tris, pH 8.0, 0.4 M NaCl, 30 mM sodium butyrate, 1 mM PMSF) [16, 17]. The cell pellets were centrifuged and resuspended, with occasional vortexing, in 0.4 N sulfuric acid (3 mL) at 4 °C for 1 h. The histones in the supernatant were precipitated by cold acetone, centrifuged, dried, and redissolved in water.

Histone H3 Isolation and Digestion

Histone H3 was isolated from the core histone mixture by HPLC on an Agilent 1100 system (Agilent Technologies, Palo Alto, CA) and a 4.6 × 250 mm C4 column (Grace Vydac, Hesperia, CA) was used. The flow rate was 0.8 mL/min, and a 60-min linear gradient of 30%–60% acetonitrile in 0.1% trifluoroacetic acid (TFA) was employed. Purified histone H3 was digested with sequencing-grade modified Arg-C (Roche Applied Science, Indianapolis, IN) at a protein/enzyme ratio (wt/wt) of 20:1 in a 100-mM ammonium bicarbonate buffer at 37 °C overnight. The digested peptides were further separated on the same HPLC system with a Zorbax SB-C18 capillary column (0.5 × 150 mm, 5 μm in particle size, Agilent Technologies), and a 60-min gradient of 2%–60% acetonitrile in 0.6% acetic acid was used. The flow rate was 10 μL/min.

Mass Spectrometry

MALDI-MS/MS measurements were performed on a QSTAR XL quadrupole/time-of-flight instrument equipped with an o-MALDI ion source (Applied Biosystems, Foster City, CA). The laboratory collision energy applied for MS/MS varied from 50 to 75 eV depending on peptide sequences and modification levels, and the collision gas was nitrogen.

LC-ESI-MS/MS experiments were carried out by coupling directly the effluent from the Zorbax SB-C18 capillary column (0.5 × 150 mm, 5 μm in particle size, Agilent Technologies) to an LTQ linear ion trap mass spectrometer (Thermo Electron Co., San Jose, CA). Isolated peptides were subjected to LC-MS/MS analysis, and we used a 60-min linear gradient of 2%–60% acetonitrile in 0.6% acetic acid delivered by the Agilent 1100 capillary HPLC pump at a flow rate of 6 μL/min. MS/MS experiments were carried out in either the data-dependent scan mode or the preselected ion mode. Helium was employed as the collision gas, and the normalized collision energy was 30%. The width for precursor ion isolation was set at 2.5 (m/z) with an activation Q of 0.25 and an activation time of 30 ms. The spray voltage was 4.5 kV, and the temperature for the heated capillary was 275 °C. The MS^3 experiments were carried out on the same instrument, where the isolation Q for the second-stage precursor ion selection was lowered to 0.23 to trap better the low-m/z fragments.

Results and Discussion

MALDI-MS/MS Suggests an Unusual Modification of Pro-16 in Human Histone H3 and the Methylation of Arg-83 in Yeast Histone H3

To assess the PTMs of histone H3, we extracted the protein from cultured human (HL-60 and MCF-7) and yeast *S. cerevisiae* cells, digested it with Arg-C, and subjected the digestion mixtures to MALDI-MS and MS/MS analyses. The MALDI-MS data showed that the peptide with residues 73-83 in human histone H3 was mostly unmodified, though mono- and dimethylation could also be detected (Figure 1a). The peptide with residues 73–83 from yeast cells as well as the peptide with residues 9–17 in histone H3 isolated from human cells were mono-, di- and trimethylated (Figure 1b and c). On the other hand, the peptide housing residues 9–17 was predominantly unmodified in H3 isolated from yeast cells (Figure 1d). In this context, it is worth noting that the ion of *m/z* 972.6 in Figure 1d is attributed to the protonated ion of another histone H3 peptide, KQLASKAAR, which was supported by MS/MS analysis (spectrum not shown).

MALDI-MS/MS analysis of the trimethylated forms of the two peptides, however, suggests the modifications of Pro-16 and Arg-83 (Figure 2 and Figure 3). In the product-ion spectrum of the $[M + H]^+$ ions of the trimethylated and monoacetylated peptide KSTGGKAPR (*m/z* 984.4, Figure 2), we observed the b_5 + 2Me, b_6 + 2Me + Ac and b_7 + 2Me+Ac ions, supporting the dimethylation of Lys-9 and the acetylation of Lys-14. We also observed the neutral loss of an $HN(CH_3)_2$ from the b_6 + 2Me + Ac and b_7 + 2Me + Ac ions, lending further support for the presence of a dimethyllysine in the peptide. The presence of an acetylated lysine in this peptide is

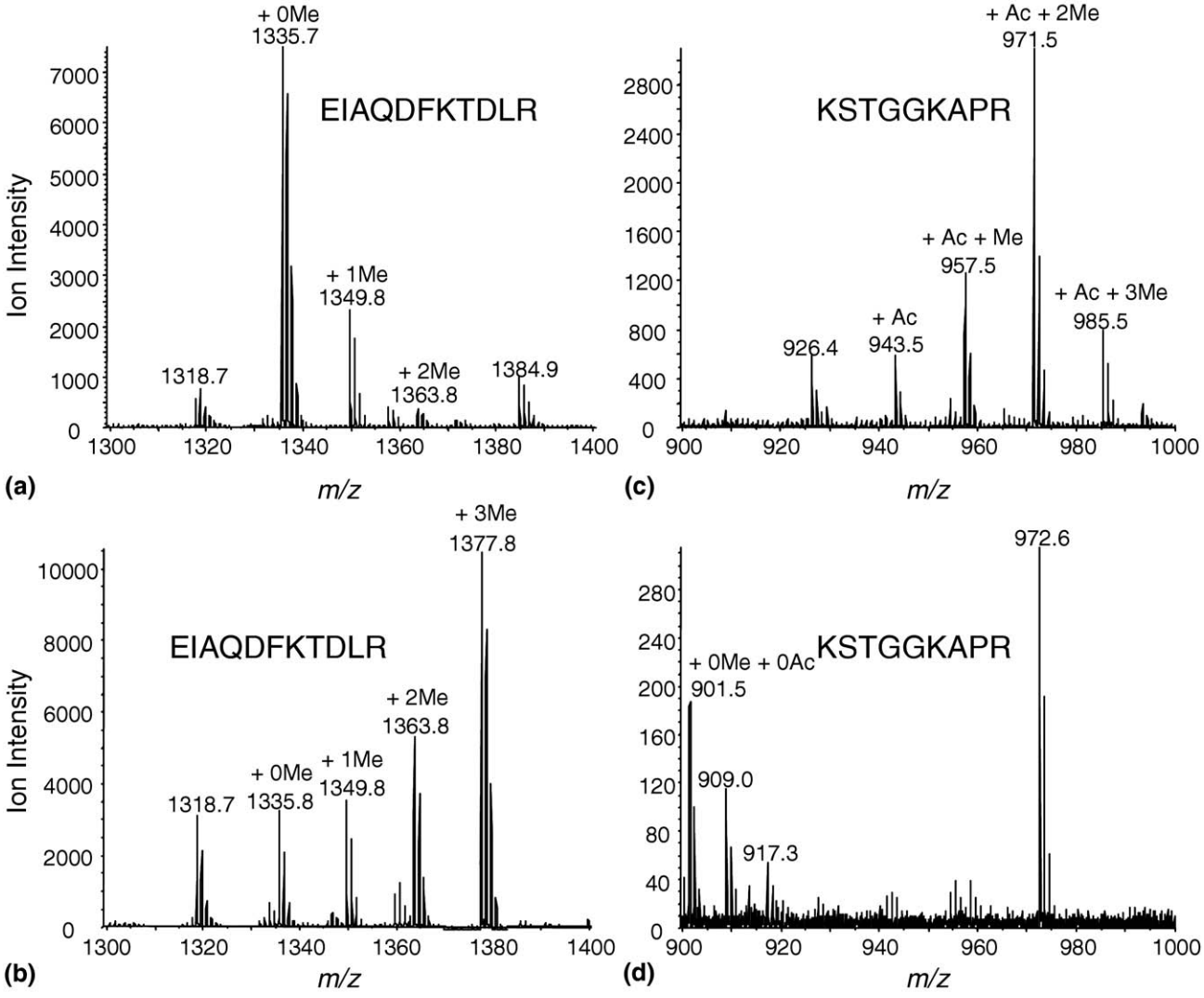

Figure 1. MALDI-MS of the Arg-C produced peptide with residues 73–83 in histone H3 isolated from MCF-7 human breast cancer cells (**a**) and yeast cells (**b**). Shown in (**c**) and (**d**) are the MALDI-MS of the Arg-C produced peptide fragment containing residues 9–17 in histone H3 extracted from MCF-7 cells and yeast cells, respectively.

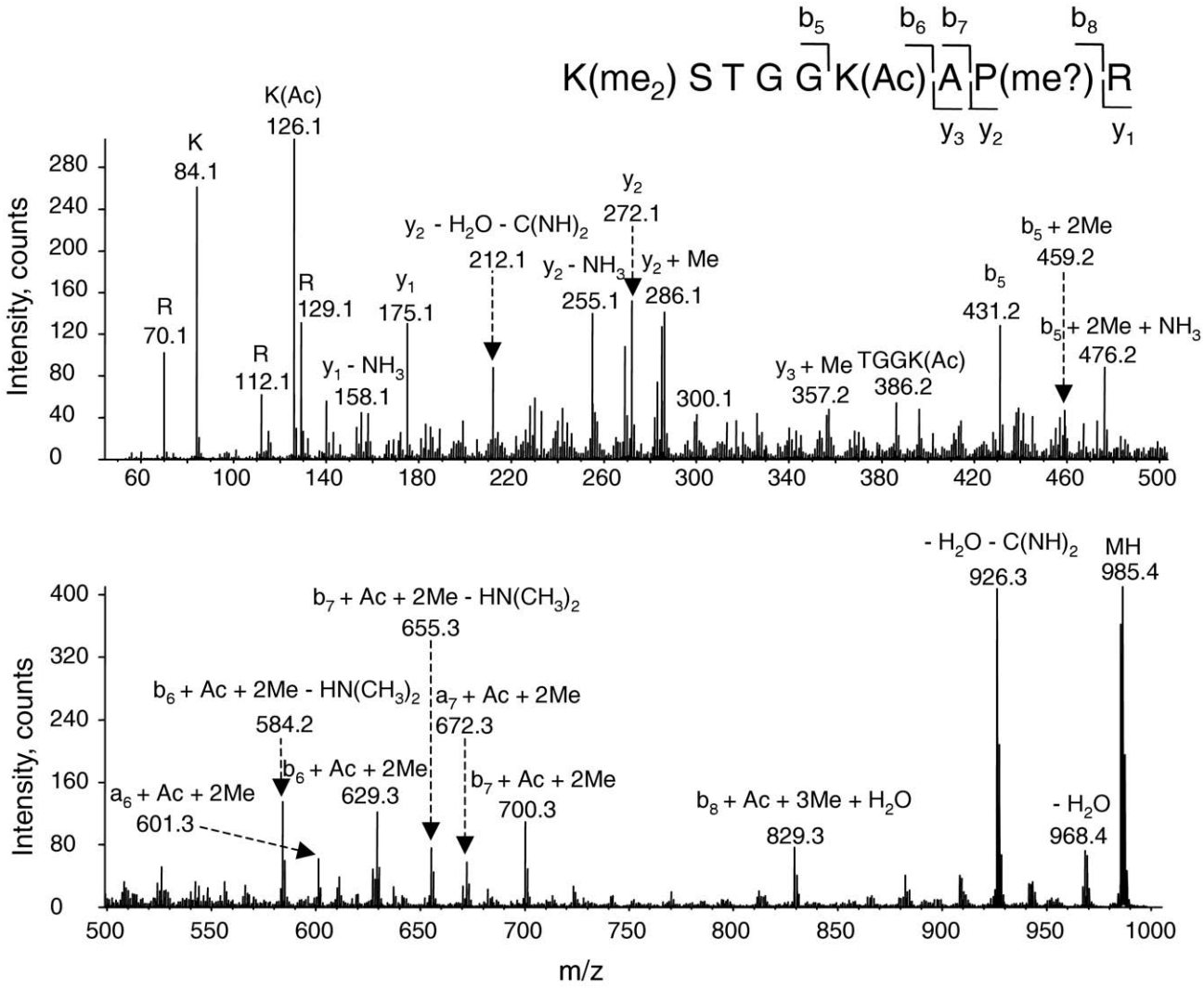

Figure 2. MALDI-MS/MS of the singly charged ion of the peptide with human H3 peptide with residues 9–17 that is monoacetylated and trimethylated (*m/z* 985.5).

also supported by the observation of the diagnostic ion of *m/z* 126.1 (Figure 2) [18]. Additionally, the observation of the y_2 + Me and y_3 + Me ions and the finding of the b_7 + Ac + 2Me and b_8 + Ac + 3Me + H_2O ions suggest that there is a covalent modification of Pro-16 giving rise to a mass increase of 14 Da (Figure 2). Monomethylation can lead to a mass increase of 14 Da; however, the methylation of internal proline has not been reported, though methylation of N-terminal proline is known [19].

In the MS/MS of the trimethylated peptide EIAQDFKTDLR (*m/z* 1377.5, Figure 3, Lys-79 was found to be di- or trimethylated, which is supported by the mass difference between the y_4 and y_5 ions. A series of y_n + Me (n = 1–2) and b_m + 2Me (m = 8–9) ions were observed, suggesting that Arg-83 might be partially monomethylated. It is worth noting that the assignments of fragment ions were consistent with exact mass measurements of fragment ions based on the MS/MS acquired on the QSTAR instrument (Table 1).

We also acquired the MALDI-MS/MS of these two peptides bearing lower levels of methylation. Methylation of Pro-16 or Arg-83 could not be detected when the overall methylation level of the peptides dropped. In the MS/MS of the dimethylated peptide with residues 9–17 (Figure S1), which can be found in the electronic version of this article, the presence of the b_n + 2Me (n = 3–8) ions supports the dimethylation of Lys-9; the absence of y_2 + Me and y_3 + Me ions, along with the presence of y_2 and y_3 ions, shows that Pro-16 was not modified (Figure S1 and S2 depict the MS/MS in the low-*m/z* range to better visualize the low-abundance fragments). Together, the above MALDI-MS/MS results suggest that Pro-16 and Arg-83 might be partially modified with a 14-Da mass increase only when these two peptides are trimethylated.

LC-ESI-MS/MS Supports the Absence of Pro-16 and Arg-83 Modification

To further assess the nature of methylation of the above peptides, we subjected the same trimethylated H3 pep-

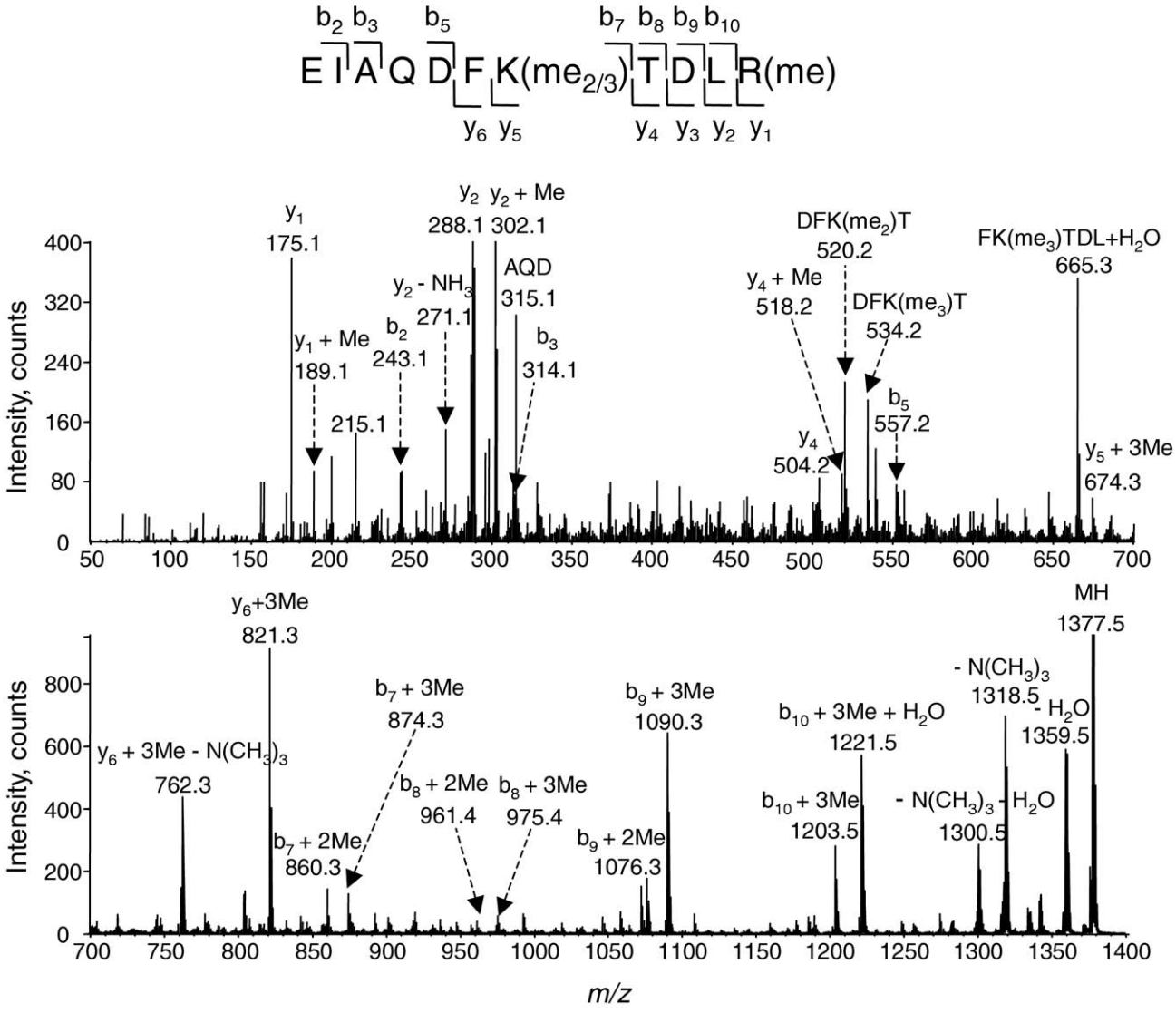

Figure 3. MALDI-MS/MS of the singly charged ion (*m/z* 1377.8) of the trimethylated peptide with residues 73–83 in histone H3 extracted from *S. cerevisiae* cells.

tides to LC-ESI-MS/MS analyses. Surprisingly, the ESI-MS/MS data did not reveal the modification on Pro-16 or Arg-83, instead they only supported the existence of a trimethyllysine in these two peptides (Figure 4).

Unlike the MALDI-MS/MS results, the ESI-MS/MS of the doubly charged H3 peptide with residues 9–17 showed the formation of y_1, y_2, y_3, and y_n + Ac (n = 4–8) ions, supporting the acetylation of Lys-14 and the lack of modification on Pro-16 (Figure 4a). Additionally, the observation of the neutral loss of a trimethylamine [$N(CH_3)_3$] from both the precursor ion and the b_2 + 3Me ion reveals the trimethylation of Lys-9 (Figure 4a).

ESI-MS/MS of the doubly charged ion of the trimethylated peptide with residues 73–83 of histone H3 isolated from yeast cells also showed the lack of methylation on Arg-83 (Figure 4b), instead the formation of abundant y_2, y_6 + 3Me, b_9 + 3Me, and b_{10} + 3Me ions supports the trimethylation of Lys-79 and the absence of methylation of Arg-83.

The ESI-MS/MS for the trimethylated peptides supports unequivocally the lysine trimethylation as previously reported [20, 21] and the absence of modification on Pro-16 or Arg-83, which is in stark contrast with the MALDI-MS/MS results. However, LC-ESI-MS/MS and MALDI-MS/MS data for these peptides with lower modification levels are consistent, which both supported the methylation of lysine residues alone. The interesting discrepancy between LC-ESI-MS/MS and MALDI-MS/MS on the trimethylated peptides calls for further investigation on the nature of methylation in these peptides.

MS/MS Analyses of Synthetic Trimethyllysine-Containing Peptide EIAQDFK(me$_3$)TDLR

To gain insights into the above disagreement between MALDI- and ESI-MS/MS results, we obtained a synthetic trimethyllysine-containing peptide bearing the

Table 1. A summary of calculated and measured *m/z* of product ions for the trimethylated human H3 peptide with residues (9–17) and yeast H3 peptide with residues (73–83). The MS/MS was calibrated by using the y_1 and precursor ions as internal references

Productions	Calculated *m/z*	Measured *m/z*	Deviation (p.p.m.)
K(me_3)STGGK(Ac)APR			
y_2	272.1717	272.1662	−20
y_2+Me	286.1874	286.1850	−8.3
y_2-NH_3	255.1452	255.1448	−1.5
y_2-H_2O-C($NH)_2$	212.1395	212.1361	−16
y_3+Me	357.2245	357.2157	−24
b_5	431.2249	431.2207	−9.7
b_5+2Me	459.2562	459.2553	−1.9
b_6+Ac+2Me	629.3618	629.3782	26
b_6+Ac+2Me-HN($CH_3)_2$	584.3039	584.3103	10
a_6+Ac+2Me	601.3668	601.3788	19
b_7+Ac+2Me	700.3989	700.4117	18
b_7+Ac+2Me-HN($CH_3)_2$	655.3410	655.3570	24
a_7+Ac+2Me	672.4040	672.4117	11
b_8+Ac+3Me+H_2O	829.4778	829.4677	−12
TGGK(Ac)	386.2034	386.2022	−3.1
MH-N($CH_3)_3$	926.5055	926.4909	−15
MH-NH_3	968.5524	968.5525	0.1
EIAQDFK(me_3)TDLR			
y_1+Me	189.1347	189.1360	6.8
y_2	288.2030	288.2006	−8.3
y_2+Me	302.2187	302.2220	10
y_4	504.2776	504.2737	−7.7
y_4+Me	518.2933	518.2992	11
y_5	632.3726	632.3731	0.7
y_5+3Me	674.4196	674.4253	8.4
y_6+3Me	821.4880	821.4685	−24
y_6+3Me-N($CH_3)_3$	762.4145	762.4182	4.8
b_2	243.1339	243.1285	−22
b_8+2Me	961.4990	961.4882	−11
b_8+3Me	975.5147	975.4958	−19
b_9+2Me	1076.5260	1076.5250	−0.9
b_9+3Me	1090.5417	1090.5233	−16
b_{10}+2Me	1189.6100	1189.6324	18
b_{10}+3Me	1203.6257	1203.6505	20
b_{10}+3Me+H_2O	1221.6363	1221.6593	18
AQD	315.1299	315.1267	−10
DFK(me_2)T	520.2766	520.2703	−12
DFK(me_3)T	534.2923	534.2894	−5.4
FK(me_3)TDL	665.3869	665.3765	−16
MH-N($CH_3)_3$	1318.6638	1318.6801	12
MH-H_2O	1359.7267	1359.7397	9.5

same sequence as the yeast H3 peptide with residues 73–83 and subjected it to MALDI- and ESI-MS/MS analyses. The ESI-MS/MS of this peptide supports the lysine trimethylation (spectrum not shown), which is in keeping with the ESI-MS/MS of the peptide of histone H3 isolated from yeast cells. Interestingly, the product-ion spectrum of the MALDI-produced singly charged ion of this trimethyllysine-harboring peptide (Figure S3) is almost the same as that of the Arg-C produced peptide from histone H3 isolated from yeast cells (Figure 3). In this context, we found y_n + Me (n = 1–2) and b_m + 2Me (m = 7–9) ions, although the C-terminal arginine in this synthetic peptide was not methylated. These results support unambiguously the lack of methylation of Arg-83 in histone H3 in yeast cells and suggest an unusual fragmentation of the MALDI-produced ions of trimethyllysine-containing peptides. In the latter respect, the above findings suggest that a methyl group may migrate from the side chain of trimethyllysine to the C-terminal side of the peptide during its fragmentation.

Charge State-Specific Migration of Methyl Group for Trimethyllysine-Carrying Peptides

To assess whether this unusual fragmentation occurs generally for trimethyllysine-containing peptides, we further obtained two synthetic, alanine-rich, and trimethyllysine-containing peptides, AAAK(me_3)AAK and AAAK(me_3)AAR, and subjected them to MALDI- and ESI-MS/MS analyses. ESI-MS/MS of the doubly charged ions of the two peptides confirmed unambiguously the trimethylation of the central lysine residue in the peptide, as manifested by the observation of b_n (n = 2–3), b_m + 3Me (m = 4–6), y_x (x = 1–3), and y_5 + 3Me ions (Figure S4a and b). However, ions arising from methyl group migration, i.e., b_m + 2Me (m = 4–6) or y_x + Me (x = 1–3), could not be detected in either spectrum.

By contrast, fragment ions emanating from the methyl group migration could be readily observed in the MS/MS of the singly charged ions of the two peptides produced by either MALDI or ESI (Figure 5 shows the ESI-MS/MS results). For instance, we observed y_n + Me (n = 1–3) and a_m + 2Me/b_m + 2Me (m = 4–6) ions in the MS/MS of the ESI-produced singly charged ion of AAAK(me_3)AAR (Figure 5a). While the above a_m + 2Me/b_m + 2Me ions could be observed in the corresponding spectrum of AAAK(me_3)AAK, the y_n + Me (n = 1–3) ions are barely detectable (Figure 5b). The different basicities of lysine and arginine, which reside on the C-termini of these two synthetic peptides, may account for their different susceptibilities in the formation of y and y + Me ions. The MALDI-MS/MS of the singly charged ions of the two peptides gave similar results (Data not shown). Together, the above results demonstrated that the interesting methyl group migration could only be observed in the MS/MS of the singly-charged ions of trimethyllysine-containing peptides, regardless of whether the precursor ions are produced by MALDI or ESI.

To further examine the destination of the migrated methyl group, we acquired the MS^3 data, which record the product ions formed from the cleavages of the y_1 + Me, y_2 + Me, and y_3 + Me ions observed in Figure 5a. As depicted in Figure 6a, the product-ion spectrum emanating from the fragmentation of the y_1 + Me ion gives the neutral losses of NH_3 (*m/z* 172), methylamine (CH_3NH_2, *m/z* 158), and monomethylcarbodiimide (HN=C=NCH_3, *m/z* 133). The latter two are characteristic neutral losses for the protonated ions of peptides

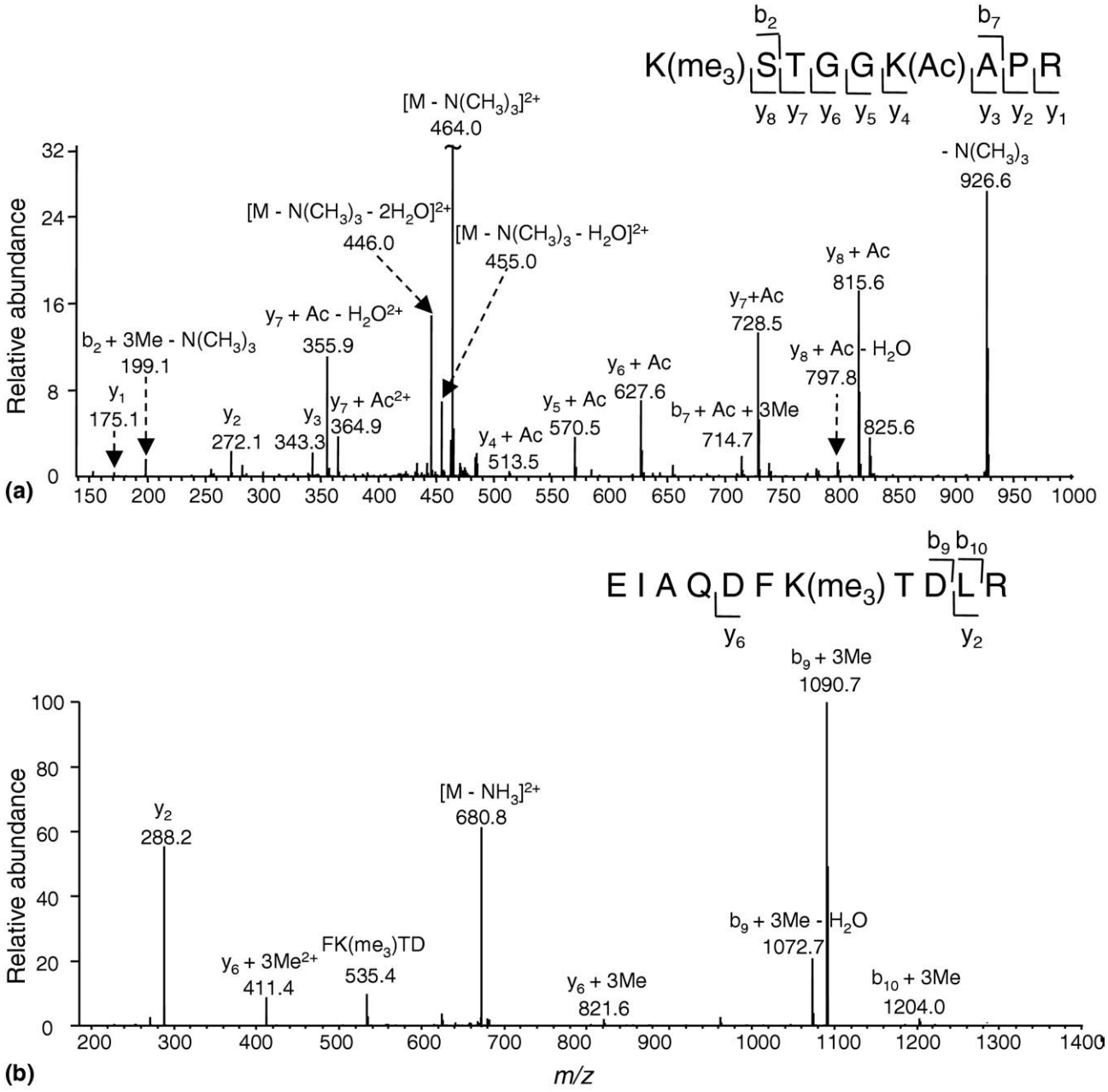

Figure 4. MS/MS of the ESI-produced doubly charged ions of trimethylated peptides with residues 9–17 in histone H3 isolated from MCF-7 cells (**a**) and with residues 73–83 in histone H3 extracted from *S. cerevisiae* cells (**b**).

housing an N^G-monomethyl-L-arginine [22–24]. Indeed the MS^3 arising from the further cleavage of y_1 + Me ion shows the formation of all fragment ions that can be found in the product-ion spectrum of the $[M + H]^+$ ion of standard N^G-monomethyl-L-arginine (Figure 6a and Figure S5). This result supports that the methyl group in the side-chain of a trimethyllysine side chain can be migrated to the guanidinium group of arginine. Aside from those ions that can be found in the MS/MS of the standard N^G-monomethyl-L-arginine, the MS^3 reveals the formation of three unique fragment ions, i.e., the ions of *m/z* 157, 130, and 60. The former two ions are attributed to the neutral loss of a CH_3OH and the combined neutral losses of NH_3 and HN=C=NH, respectively, whereas the ion of *m/z* 60 is attributed to the protonated ion of the guanidinium moiety of arginine. The formation of these three ions is consistent with the view that a portion of the y_1 + Me ion carries the methyl group on the C-terminal carboxylic acid functionality. The relatively low abundances of these three fragment ions suggest that the methyl group in y_1 + Me ion resides mainly on the guanidinium portion of arginine.

The MS^3 of the y_2 + Me and y_3 + Me ions both revealed the formation of abundant y_1 + Me (*m/z* 189) and y_1 +

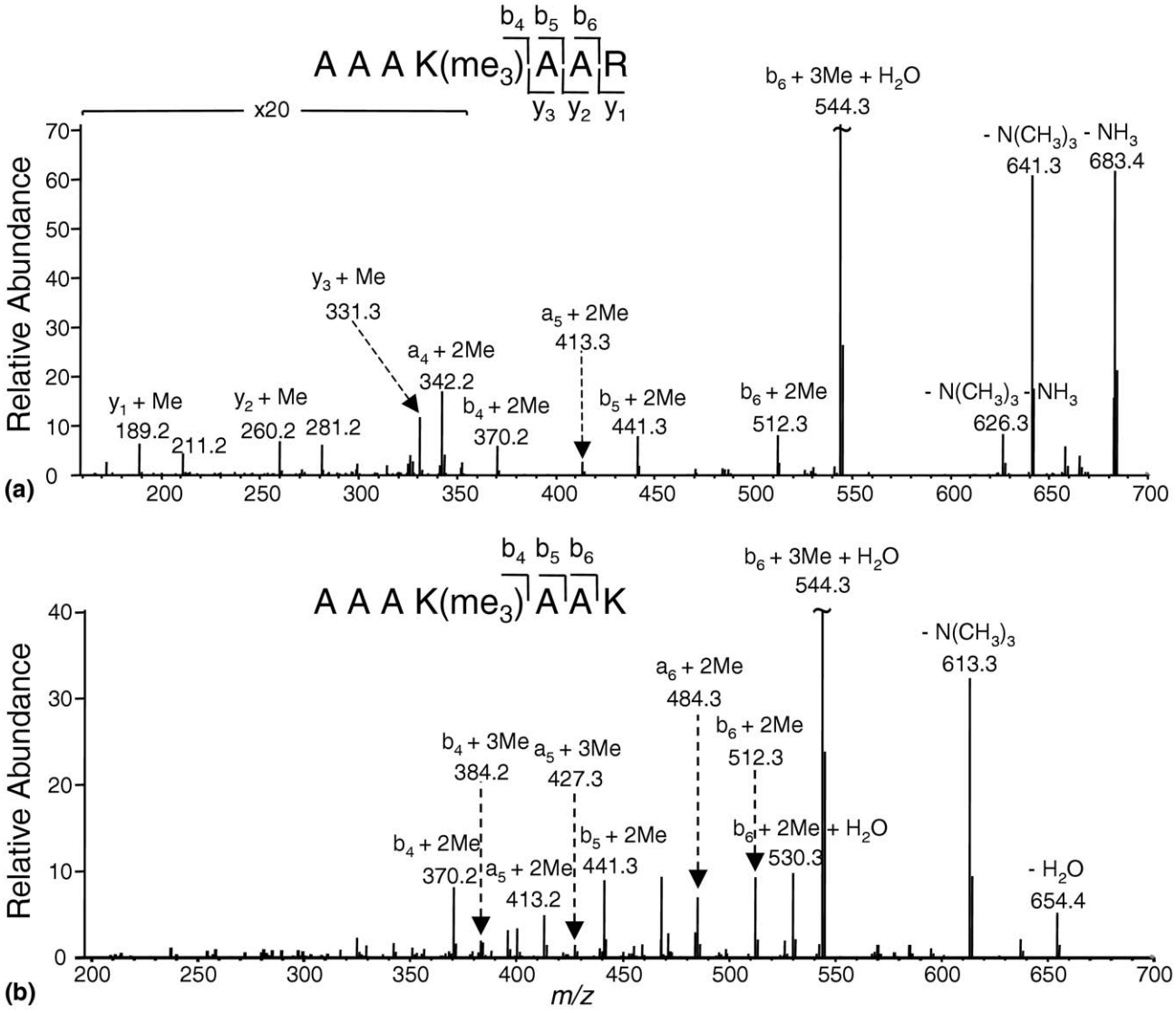

Figure 5. MS/MS of the ESI-produced singly charged ions of the synthetic, trimethyllysine-bearing peptides AAAK(me$_3$)AAR (*m/z* 700.5) (**a**) and AAAK(me$_3$)AAK (*m/z* 672.5) (**b**).

Me-NH$_3$ (*m/z* 172) ions, whereas the unmethylated y$_1$ ion was not detectable (Figure 6b and c), strongly suggesting that in these two fragment ions, the methyl group is located on the C-terminal arginine residue. It is worth noting that the above MS^3 results also lend further support of our assignments of the y$_n$ + Me (n = 1–3) ions observed in Figure 5a.

During the fragmentation of protonated ions of peptides induced by CID or SID, a "mobile proton" can be transferred to the amide linkage to induce the formation of the b and its complementary y ions [7–13]. This model is successful in rationalizing the formation of b and y ions and the preference of specific chain cleavage sites during the CID or SID of peptides through the "charge directed" pathway. Here we showed that, during the collisional activation of the singly charged, trimethyllysine-harboring peptides, regardless of whether the ion is produced by MALDI or ESI, a methyl group can migrate from the side-chain of a trimethyllysine to the guanidinium side-chain or the carboxylic acid moiety of the C-terminal arginine. This methyl group migration does not occur during the fragmentation of the ESI-produced doubly charged ions of trimethyllysine-containing peptides.

The difference between MALDI-MS/MS and LC-ESI-MS/MS results on the tri-methylated peptides with residues 9–17 and 73–83 is attributed to the peptide charge difference. While the MALDI-produced ions of these peptides carry only one charge, the ESI-formed doubly charged ions of these two peptides adopt a protonated side chain or N-terminus other than the charge located on the side-chain of the trimethylated lysine residue. These protons are mobile, thereby fulfilling the requirement for the "charge-directed" pathway. Peptide

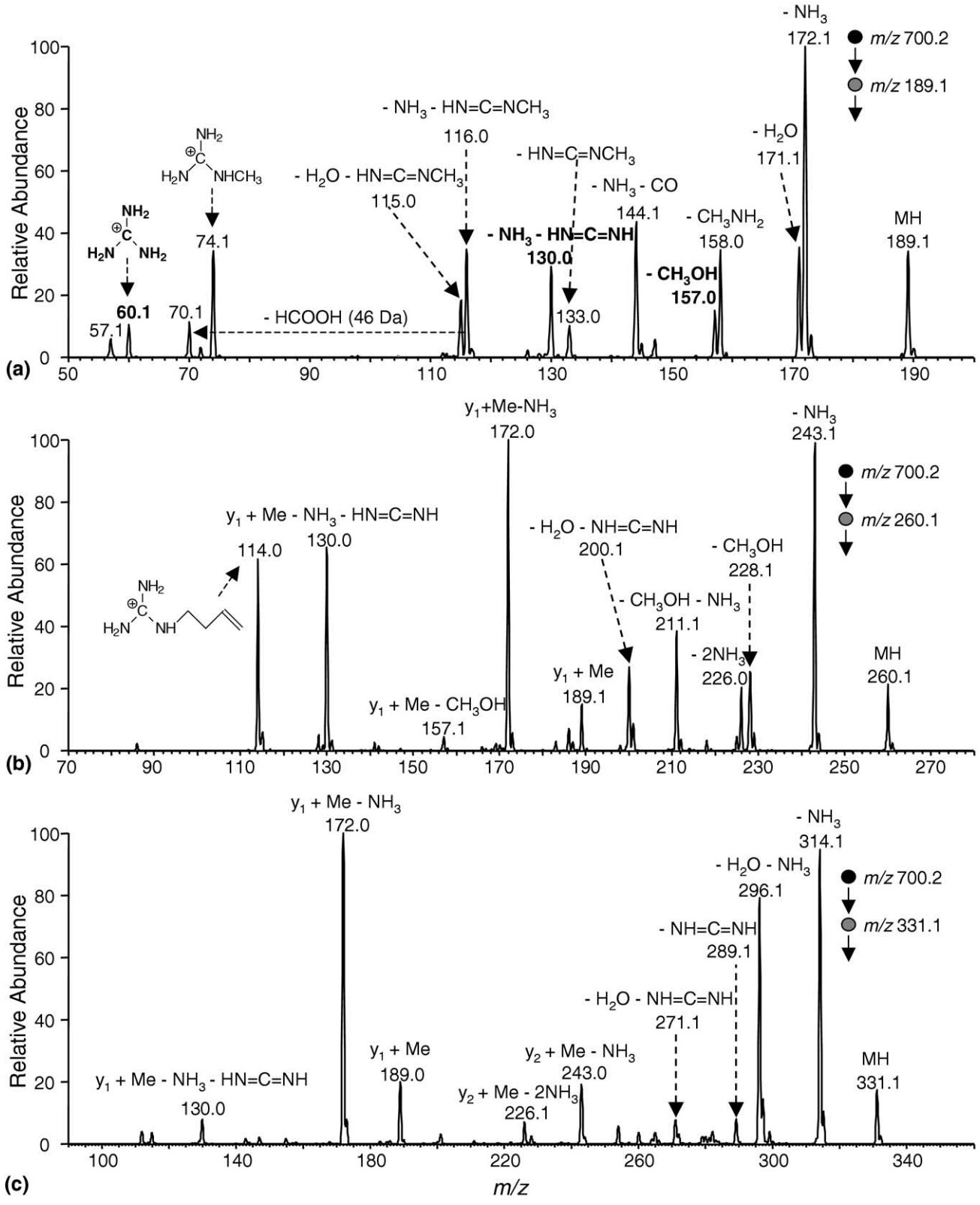

Figure 6. MS^3 monitoring the fragmentation of the y_1 + Me (**a**), y_2 + Me (**b**), and y_3 + Me (**c**) ions observed in Figure 5a. In Figure 6a, the three fragment ions that were not observed in the MS/MS of the $[M + H]^+$ ion of N^G-monomethyl-L-arginine (Figure S5) are labeled in bold fonts.

bond cleavage arising from the mobile proton transfer is likely to be energetically more favorable than the methyl group migration. Thus, we did not observe fragment ions emanating from the methyl group transfer in the MS/MS of the ESI-produced doubly charged ions of the trimethyllysine-containing peptides.

MALDI-MS/MS and LC-ESI-MS/MS for the peptides with lower methylation level are consistent because these peptides carry a "mobile" proton to facilitate the fragmentation, as observed for the fragmentation of the ESI-produced doubly charged ions of the trimethyllysine-housing peptides where the transfer of a mobile proton takes place. It is worth emphasizing that the methyl group migration is not essential for the fragmentation of trimethylated lysine-containing peptides. The formation of y_n ($n = 1, 2$ in Figures 2, 3, and S3) ions supports that the cleavage of amide bonds can occur in the absence of methyl group transfer.

Conclusions

We observed an interesting methyl group transfer phenomenon during the fragmentation of singly charged ions of trimethyllysine-containing peptides; a methyl group on the side chain of trimethyllysine can be transferred to the C-terminal residue of the peptide before amide bond cleavage. The results from the present study call for special attention to the possibility of this type of methyl group migration while MS/MS of singly charged ions is employed to interrogate the methylation of proteins, including histones.

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Appendix A Supplementary Material

Supplementary material associated with this article may be found in the online version at doi:10.1016/j.jasms.2009.02.014.

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